=> d his

(FILE 'HOME' ENTERED AT 14:05:03 ON 23 JUN 2004)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DISSABS, DDFB, DDFU, DGENE, DRUGB, DRUGMONOG2, ...' ENTERED AT 14:07:02 ON 23 JUN 2004

SEA	GLUCOAMYLASE
1	FILE ADISCTI
2	FILE ADISINSIGHT
700	FILE AGRICOLA
41	FILE ANABSTR
10	FILE AQUASCI
526	FILE BIOBUSINESS
48	FILE BIOCOMMERCE
2296	FILE BIOSIS
2085	FILE BIOTECHABS
2085	FILE BIOTECHDS
709	FILE BIOTECHNO
618	FILE CABA
29	FILE CANCERLIT
5385	FILE CAPLUS
633	FILE CEABA-VTB
3	FILE CEN
29	FILE CIN
62	FILE CONFSCI
1	FILE CROPB
96	FILE DISSABS
22	FILE DDFB
23	FILE DDFU
1270	FILE DGENE
22	FILE DRUGB
39	FILE DRUGU
12	FILE EMBAL
1041	FILE EMBASE
562	FILE ESBIOBASE
9	FILE FEDRIP
2	FILE FOMAD
32	FILE FOREGE
461	FILE FROSTI
1396	FILE FSTA
549	FILE GENBANK
2	FILE HEALSAFE
540	FILE IFIPAT
802	FILE JICST-EPLUS
937	FILE LIFESCI
1007	FILE MEDLINE
2	FILE NIOSHTIC
29	FILE NTIS
3	FILE OCEAN
1347	FILE PASCAL
2	FILE PHAR
2	FILE PHIN
68	FILE PROMT
2	FILE PROUSDDR
1	FILE RDISCLOSURE
2119	FILE SCISEARCH
481	FILE TOXCENTER
5199	FILE USPATFULL
136	FILE USPAT2

```
                1    FILE VETU
              921    FILE WPIDS
                2    FILE WPIFV
              921    FILE WPINDEX
L1               QUE GLUCOAMYLASE
                 ---------

     FILE 'CAPLUS, BIOSIS, SCISEARCH, BIOTECHDS, FSTA, PASCAL, EMBASE,
     MEDLINE, LIFESCI, WPIDS, JICST-EPLUS, BIOTECHNO, AGRICOLA' ENTERED AT
     14:08:08 ON 23 JUN 2004
L2               1 S L1 AND (POSITION 402 OR AMINO ACID 402 OR TYR402 OR Y402)
```

L6 ANSWER 143 OF 143 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1991-01384 BIOTECHDS
TITLE: Expression and routeing of human lysosomal alpha-glucosidase in transiently transfected mammalian cells; **glucoamylase** gene cloning and expression in COS-1 and HeLa cell culture; potential application in glycogenosis type-II gene therapy
AUTHOR: Hoefsloot L H; Willemsen R; Kroos M A; Hoogeveen-Westerveld M; Hermans M M P; *Reuser A J J
LOCATION: Department of Cell Biology and Genetics, Erasmus University, P.O. Box 1738, 3000 DR, Rotterdam, The Netherlands.
SOURCE: Biochem.J.; (1990) 272, 2, 485-92
CODEN: BIJOAK
DOCUMENT TYPE: Journal
LANGUAGE: English
AB cDNA encoding human acid alpha-glucosidase (**glucoamylase**, EC-3.2.1.3) was cloned in vector plasmid pSG5 and used to transform COS-1 and HeLa cells. mRNA was synthesized from the phage T7 promoter in front of the cDNA insert, and used to direct protein synthesis in a reticulocyte translation system. Only translation of sense mRNA led to protein production. Plasmid pSHAG1 did not encode a functional enzyme, due to an Arg residue replacing a Trp residue at **position 402**. The mutation did not affect enzyme production, but interfered with post-translational modification and intracellular transport of the precursor. Pulse-chase experiments suggested that the precursor was denatured. A Trp-402-containing enzyme (encoded by plasmid pSHAG2) was processed properly, was active, and reached the membrane and the medium. The proteins formed in the absence and in the presence of microsomes corresponded in their mol.weight to previously identified unglycosylated and glycosylated precursors of **glucoamylase**, obtained by translation in vitro of total RNA from human fibroblasts. The enzyme may be useful in gene therapy of e.g. glycogenosis type II. (36 ref)

=> d l2 ibib ab

L2 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1991-01384 BIOTECHDS
TITLE: Expression and routeing of human lysosomal alpha-glucosidase in transiently transfected mammalian cells; **glucoamylase** gene cloning and expression in COS-1 and HeLa cell culture; potential application in glycogenosis type-II gene therapy
AUTHOR: Hoefsloot L H; Willemsen R; Kroos M A; Hoogeveen-Westerveld M; Hermans M M P; *Reuser A J J
LOCATION: Department of Cell Biology and Genetics, Erasmus University, P.O. Box 1738, 3000 DR, Rotterdam, The Netherlands.
SOURCE: Biochem.J.; (1990) 272, 2, 485-92
CODEN: BIJOAK
DOCUMENT TYPE: Journal
LANGUAGE: English
AB cDNA encoding human acid alpha-glucosidase (**glucoamylase**, EC-3.2.1.3) was cloned in vector plasmid pSG5 and used to transform COS-1 and HeLa cells. mRNA was synthesized from the phage T7 promoter in front of the cDNA insert, and used to direct protein synthesis in a reticulocyte translation system. Only translation of sense mRNA led to protein production. Plasmid pSHAG1 did not encode a functional enzyme, due to an Arg residue replacing a Trp residue at **position 402**. The mutation did not affect enzyme production, but interfered with post-translational modification and intracellular transport of the precursor. Pulse-chase experiments suggested that the precursor was denatured. A Trp-402-containing enzyme (encoded by plasmid pSHAG2) was processed properly, was active, and reached the membrane and the medium. The proteins formed in the absence and in the presence of microsomes corresponded in their mol.weight to previously identified unglycosylated and glycosylated precursors of **glucoamylase**, obtained by translation in vitro of total RNA from human fibroblasts. The enzyme may be useful in gene therapy of e.g. glycogenosis type II. (36 ref)

Display Abstract Show: 20 Sort Send to Text

1: Biochem J. 1990 Dec 1;272(2):485-92. Related Articles, L

Expression and routeing of human lysosomal alpha-glucosidase in transiently transfected mammalian cells.

Hoefsloot LH, Willemsen R, Kroos MA, Hoogeveen-Westerveld M, Hermans MM, Van der Ploeg AT, Oostra BA, Reuser AJ.

MGC-Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands.

Previously isolated lysosomal alpha-glucosidase cDNA clones were ligated to full-length constructs for expression in vitro and in mammalian cells. One of these constructs (pSHAG1) did not code for functional enzyme, due to an arginine residue instead of a tryptophan residue at amino acid position 402. The mutation does not affect the rate of enzyme synthesis, but interferes with post-translational modification and intracellular transport of the acid alpha-glucosidase precursor. Using immunocytochemistry it is demonstrated that the mutant precursor traverses the endoplasmic reticulum and the Golg complex, but does not reach the lysosomes. Pulse-chase experiments suggest premature degradation. The Trp-402-containing enzyme (encoded by construct pSHAG2) is proces properly, and has catalytic activity. A fraction of the enzyme is localized at the plasma membrane. It is hypothesized that membrane association of the acid alpha-glucosidase precursor, as demonstrated by Triton X-114 phase separation, is responsible for transpor this location. Transiently expressed acid alpha-glucosidase also enters the secretory pathway, since a catalytically active precursor is found in the culture medium. This precursor has the appropriate characteristics for use in enzyme replacement therapy. Efficient uptake via the mannose 6-phosphate receptor results in degradation of lysosom glycogen in cultured fibroblasts and muscle cells from patients with glycogenosis type II

PMID: 2268275 [PubMed - indexed for MEDLINE]

Display Abstract Show: 20 Sort Send to Text